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THE SPORE COATS OF SELAGINELLA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXX.

FLORENCE LYON.

(WITH PLATES X AND XI)

SINCE the development of the modern technique in botany, comparatively little has been done in investigating the mode of formation of cell walls or of spore coats, and botanists are still much in the dark as to the nature, as well as the morphology, of certain complex structures protecting the protoplasm of spores. A recent contribution to this subject by FITTING[1] is of special importance, because as a result of his study of the development of the spore coats of certain species of Selaginella, he advanced a theory that they grow quite independently of the protoplasm of the spore, with which during the time of their greatest increase in bulk they have no organic connection except at one point. This, of course, is revolutionary and quite at variance with all previous ideas of the growth of membranes; and if it is true that protoplasm "can act at a distance," and without organic connection can build massive structures, the fact is of the greatest importance.

At the time that FITTING'S paper appeared, I was investigating certain phases in the life history of two native species of Selaginella—*S. apus* and *S. rupestris*. In the main the peculiar phenomena connected with the formation of the spore coats of the first named species seemed to agree with those described by FITTING; but the development of the spores of *S. rupestris* differed so completely that it became a matter of interest to determine whether there were two distinct types of spore formation in this genus or whether the differences were more apparent than real. The present work was undertaken with a view to throwing some light upon this question. The difficulties which all investigators have encountered at every stage of technique, fixing, embedding, sectioning, and staining, together with the fact that Selaginella spores are *sui generis*, and one has no standard of comparison, make one fearful of drawing conclusions from artifacts. Apparently the only way to lessen this danger and make results convincing, is to have a very large number of preparations killed and treated in various ways, for little critical study can be made of the living spore. This has made my investigation an exceedingly tedious one, for in the early development of the spore, transformation takes place so rapidly that important phases are obtained with difficulty, and if not found the danger of misinterpretation is very great. The results of my work fail to confirm FITTING'S theory, nor am I able to accept his description of the origin of the coats for the species that I have investigated. It is quite certain, I think, that the spore coats of *S. rupestris* do not lose their complete organic connection with the protoplast during growth, and I have a growing conviction that to our imperfect technique are due the phenomena which gave rise to FITTING'S theory and were described by FITTING, CAMPBELL, and all other workers in this group, including myself.

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[1] FITTING, HANS, Bau und Entwickelungsgeschichte der Makrosporen von Isoetes und Selaginella und ihre Bedeutung für die Kenntniss des Wachsthums pflanzlicher Zellmembranen. Bot. Zeitung 58[1]: 107–164. 1900.

Very complete series of three species—*S. apus*, *S. Emmeliana*, and *S. rupestris*—from the origin of the sporangium to the development of the embryo sporophyte have been studied. Throughout its history *S. rupestris* is aberrant, and proves to be a plant of much importance and interest, as it has characteristics supposed to be peculiar to the seed plants. Normally, and in a greater number of cases, only two megaspores were found to develop in a sporangium. These may be two of a tetrad, the others failing to develop after the division of the mother cell; or, more important still, the mother cell may divide only into two spores. These megaspores are not shed from the megasporangium at all! The female gametophytes and the young sporophytes are retained in the old strobilus until the latter have roots, stems, and leaves; then the tissue of the sporangium and megasporophyll decays, thus liberating the bodies that lack only integuments to be seeds. A still further reduction of megaspores to *one* is infrequently found. In tracing the growth of the spores, I again emphasize the fact that at no time is there any appearance which would suggest that the protoplasm is not in contact *at every point* with the thick gelatinous membrane from which the coats are formed.

The megaspore mother cell in all species may be distinguished from the other sporogenous cells (*fig. 1*) at the time that the sporangium wall is differentiated into three layers. It is slightly larger, more granular, and stains intensely as contrasted with its sister cells. Its wall thickens slightly, stretching and probably growing somewhat, and persists until the enclosed spores are more than half grown. I shall refer to it as the *mother cell membrane*, to distinguish it from a second membrane, the *spore membrane*, that shortly appears upon its inner surface in contact with the protoplasm (*fig. 1*). The mother cell membrane remains comparatively thin; whereas the spore membrane is gelatinous in nature, becomes very massive, and dissolves readily in many of the fixing reagents, especially in very young stages. It increases rapidly in bulk, forming a thick layer about the protoplast which for a period grows imperceptibly, if at all (*fig. 2*). As the mother cell divides, it assumes a dumb-bell shape, the two resulting spores often not completely separating from each other until half grown (*fig. 3*). At this early stage, each spore consists of a protoplast with a relatively large nucleus, and an envelop of thick gelatinous matter, the spore membrane. The two spores lie within the mother cell membrane. Whether any part of the spore membrane is formed in connection with a nuclear plate at the time of cell division could not be determined, owing to the diminutive size of the spindles and their infrequency in the material investigated. The protoplast becomes vacuolate. Many small vacuoles ultimately coalesce to form a very large one centrally placed, so that the protoplast comes to be a delicate vesicle (*fig. 4*).

The first indication of a coat is detected as a transformation of a portion of the gelatinous spore membrane. A clear homogeneous region at some little distance from the protoplast stands out in contrast to the rest of the membrane, which has become granular (*fig. 4*). This area soon becomes convoluted upon its outer surface, and these convolutions coincide with radiations in the outer granular portion of the membrane that suggest fixed diffusion currents (*fig. 4*) of solutions passing through the two membranes from the sporangium. At this time, the sporangium is turgid with fluid made up of disorganizing sporogenous cells, and a secretion poured out by the tapetum, which is the source of nourishment of the two young spores.

The spore membrane itself is a somewhat viscid substance, almost fluid in character, that flows out of the hole if a living sporangium be pricked with a needle. The spore membrane becomes much thicker at the base of the spore, so that the remarkably small protoplast lies near the apex (*fig. 5*). The growth and differentiation of the spore membrane into regions are extraordinarily rapid. Just inside of the clear area described above, a part of the membrane is much denser than elsewhere. These two regions, *i. e.*, the clear and the denser (*fig. 5*), constitute the preliminary stage of the first or outside coat, the *exospore*. It is possible in a close series of preparations to note gradual changes in structure as the coat matures. Chemical tests to determine the nature of the transforming spore membrane and of the resultant coats were very unsatisfactory. In all early stages the minute living spores are so difficult to manipulate that no reliable results could be obtained. On the other hand, material that is sectioned and upon the slide where it can be handled has already been subjected to the action of many reagents. The clear area, or outer layer of the exospore (*fig. 6*), is gradually transformed into a granulated condition, while the inner layer and the rest of the spore membrane becomes reticulated. All the regions are quite distinct and react differently to the stains used.

Up to this time the protoplast has not altered in size or density, but now it suddenly dilates—like a bubble which is blown up—and presses outward toward the exospore. When its diameter is about half that of the entire spore, a second delicate coat. the *endospore*, may be detected, having formed upon the outer surface of the protoplast. This is difficult to demonstrate unless the coat and protoplast become torn apart and displaced; for it is little more than a faint line in section during the entire period that the protoplast is expanding and pressing it back against the outer coat. The portion of the spore membrane (*fig. 6*) which is not directly transformed into the exospore, and against which the protoplast presses (*fig. 7*), disappears as the protoplast and endospore dilate. As the exospore has been undergoing modifications (*figs. 7* and *8*, *ee*) during this time, which render it more impervious to the entrance of solutions from the sporangium, this undifferentiated portion of the membrane in its turn has probably served for nutriment or has been taken into the protoplast. Often, however, a little of the original membrane may be detected crowded back against the exospore, forming a layer that easily may be misinterpreted as an additional coat, if intervening stages have been overlooked (*fig. 8, h*).

As the spores now nearly fill the sporangium cavity and the tapetal cells are disorganized, all future growth must be at the expense of the central vacuole. The principal changes in the maturing of the spore are the increase in the amount of protoplasm, with marked growth of the nucleus, and the thickening of the endospore by successive laminae upon its inner surface, which is in contact with the protoplast at first only in the region of the nucleus (*fig. 9*). Eventually, however, it becomes a layer of equal thickness throughout (*fig. 10*). The exospore is elaborately bossed and sculptured, and the protuberances form a layer—the so-called perinium—that stains quite differently from the part in contact with the endospore (*figs. 7-10*). It seems to me from its formation that it should not be regarded as a distinct coat, however, but as the outer region of the exospore. The central vacuole throughout the spore development is filled with a transparent fluid, which in early stages disappears in preparing the slide, but which becomes a coagulable mass as the female gametophyte forms (*fig. 11*).

The most conspicuous differences between *S. Emmeliana* or *S. apus* (taken as a type) and *S. rupestris*, described above, are due I think to the *simultaneous* transformation of the spore membrane of the former two coats, and to certain phenomena due to mechanical strains that arise between these two coats and the protoplast. In *S. Emmeliana*, as in all other species hitherto studied, the mother cell normally divides into a tetrad. The same difficulty arose as in the preceding species about determining the origin of that portion of the spore membrane on the contiguous surfaces (*fig. 14*). Earlier stages show the formation of nuclear plates, and there is no such appearance of lobing of the spore mother cell as in *S. rupestris*. At first the spore membrane is homogeneous and translucent (*fig. 15*), but when its thickness is about half the diameter of the protoplast (*fig. 16*), a portion of it lying in contact with the protoplast stains differently and marks the beginning of the two coats. This layer, almost as soon as it can be detected at all, shows a dual nature. It appears in section as a shaded band not sharply marked off from the undifferentiated portion of the spore membrane. Its outer region stains more vividly than the inner, perhaps because it is denser. Gradually a difference in structure becomes apparent, which I have attempted to show in *fig. 17*.

The spores are so small, stain so intensely during this critical stage, and the changes in the metamorphosing spore membrane occur with such rapidity that it has been with difficulty that I could demonstrate to my own satisfaction that one coat was not formed after the other, but both simultaneously. FITTING thinks that the endospore is formed after the first and arises *de novo* as a new formation of the protoplast. The spore is much smaller, and the changes much more rapid in this form; consequently they are harder to follow than in *S. rupestris*. The outer denser region with its roughened surface becomes the exospore, the inner the endospore. The protoplast becomes a vesicle by the formation of one large central vacuole. It is at this time when there are two coats of distinctly different structure that the peculiar phenomena occur which led FITTING to draw the conclusion that the protoplast is not concerned in the conspicuous growth of the spore coats.

As the spore grows larger, the outer coat increases in diameter and thickness much more rapidly than the inner, producing a strain in the viscid mass of which the two are concentric layers. FITTING thinks this results normally in a complete separation of the two, thus developing between them a comparatively large empty space. In a like fashion, due to the fact that the protoplast is quiescent and the endospore growing, a second "empty space" is formed between them, except at one point, thus severing all organic connection of the protoplast with the developing coats except at this point of tangency. There is no doubt that the majority of preparations have precisely this appearance. Moreover, the living spore examined in a normal salt solution seems to confirm FITTING's view of a separation of the coats, and on this fact he lays great stress. The spaces, according to him, are afterward partially filled with a solution which has entered the spore from the sporangium.[2] I have come to believe, however, that where actual gaps occur they are due to shrinkage and the dissolution wholly or in part of the intervening substance, and that the appearance of separation in the living spore can be explained by the different density of the concentric layers. In material showing no shrinkage elsewhere, I found that these regions were not empty, but completely filled with matter closely resembling the spore membrane at different periods of its transformation into coats.

It must be understood that the entire substance of the spore outside of the protoplast is a viscous mass of the consistency of thin syrup, in which two regions are becoming not only different in their physical but also in their chemical natures. This substance retains a plastic condition until the spore has attained its full size and the two coats their entire bulk. Then follow the changes which result in their hard horny or woody character. Into the viscid mass are passing solutions from the sporangium. The direct physical effect upon this mass as a whole would be a tendency to increase in diameter and bulk; but apparently all regions do not swell alike, the more resistant regions where coats are forming become drawn apart, having between them a stretched area of less dense nature, very easily dissolved by reagents, subject to great shrinkage and liable to be torn and not readily stained. From material fixed in bichromate of potassium and exposed to light, the gelatinous nature of the substance in these regions was clearly demonstrated. They always stain like the undifferentiated part of the spore membrane. FITTING also remarks that "sie verhalten sich bei allen Arten gegenüber Rengentien wie die Special Mutterzellmembranen."

There seem to be three possibilities: (1) FITTING'S view that the regions between protoplast and under coat, and between inner and outer coat, are spaces filled with a solution—comparable to a vacuole; (2) that possibly there are protoplasmic connections between the protoplast and the two coats, and that the intervening substance is protoplasm, penetrating the endospore and extending to the exospore; (3) the suggestion, made above, that normally there are no clefts in the spore, but that the matter outside of the protoplast is to be regarded as an envelop of gelatinous matter in process of local transformation into concentric layers of different physical nature, and at the same time increasing enormously in size by the imbibition of matter from the sporangium. Much time was expended in trying to demonstrate protoplasmic continuity through the coats but without success.

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[2] "Die Zwischenräume zwischen den Häuten und zwischen dem Mesospor und dem Plasma sind an lebenden Sporen ganz hyalin. . . . An fixirtem und mit dem Mikrotom geschnittenem Material sind in ihnen Gerinnungsmassen, je nach dem Entwickelungsstadium in verschiedener Menge, vorhanden. Wenn die Abhebung der Membranen gerade begonnen hat, ist von ihnen noch nichts zu sehen, erst wenn die Hohlräume etwas grösser geworden sind, werden in ihnen kleinere und grössere, hier und da netzartig verbundene sichtbar die während der Vergrösserung der Sporen schnell vermehrt werden. In noch älteren Stadien bilden sie . . . . sowohl zwischen Exospor und Mesospor, wie auch zwischen diesem und der Plasmablase je eine hyaline, glasartig durchsichtige Masse, die die beiden Räume meist nicht vollständig ausfüllt."

The third view grows convincing, as it *is* possible to obtain sections which show the spores to be a solid mass without spaces, the regions of the developing coats not being sharply marked off from the substances that FITTING regards as foreign matter, and that I believe to be merely stretched areas of the gelatinous membrane out of which the spore coats are differentiating. Indirect evidence is afforded by the growth of the coats in *S. rupestris* in which there is at no time any suggestion of "spaces filled with nutrient solutions."

The further development of the megaspore consists in the expansion of the protoplast which soon overtakes the endospore, which in turn is carried along and stretched against the exospore. The portion of the exospore in contact with the endospore (*fig. 18*) usually fails to coalesce completely with the outer part (*figs. 18* and *19*), and if one has failed to follow its behavior closely might readily be interpreted as an intermediate distinct coat. This account fails to agree with FITTING'S and CAMPBELL'S.[3] The two coats I have shown in *figs. 17–19* they call exospore and mesospore. The endospore they describe as arising *de novo* after the protoplast is fully expanded, and in contact with it, thus making *three* distinct coats. In the species which I have investigated—*S. rupestris*, *S. apus*, *S. Emmeliana*, *S. densa*, *S. cinerascens*, and two unnamed species from Jamaica—I can demonstrate only two distinct coats. May it not be possible that others have made the same error that I did in my former paper[4] when I interpreted the inner region of the exospore as a separate membrane?

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[3] CAMPBELL, D. H., Studies in the gametophyte of Selaginella. Ann. Botany **16**:419–4 8. *pl. 19.* 1902.

In a late paper by BEER,[5] the author states that he has observed a separation of coats in the pollen grains of certain Onagraceae that is quite comparable to the condition described by FITTING as occurring normally in the megaspores of certain species of Selaginella. His forthcoming paper on the subject will be one of interest, especially if he finds evidence to support FITTING'S view of protoplasm acting at a distance.

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EXPLANATION OF PLATES X AND XI.

In all figures, *a* indicates mother cell membrane; *b* the protoplast, *c* the spore membrane, *d* the nucleolus, *n* the nucleus, and *v* the vacuole.

PLATE X. Selaginella rupestris.

FIG. 1. Median section of megaspore mother cell with three sporogenous cells that fail to form spores. ×1250.

FIG. 2. Not quite median section of megaspore mother cell showing slight lobing, before division into two spores. ×1250.

FIG. 3. Nearly median section of two megaspores not completely separated from each other; that portion of the spore membrane between the two protoplasts is apparently formed by the lobing of the mother cell and not in connection with a nuclear plate. ×1250.

FIG. 4. Median section of megaspore after the formation of a large central vacuole and at the time the spore membrane shows a region differentiating into the exospore or outside coat; *e*, region differentiating into exospore; *f*, possibly diffusion currents of solution passing into spore from sporangium. ×600.

FIG. 5. Sections of two megaspores lying near the sporangium wall; by greater growth of the spore membrane at one side the protoplast has come to lie near the apex of the spore; the spore *A* is in nearly median section, *B* is not; *e*, exospore, *s*, sporogenous cells that failed to divide into megaspores. ×600.

FIG. 6. Portion of median section of megaspore (drawing not completed at base); to show further differentiation of spore membrane; *e*, exospore showing three different regions. ×600.

FIG. 7. Apical portion of median section of more advanced stage of megaspore to show the first appearance of the second coat—endospore—and the changes in the exospore; the undifferentiated part of the spore membrane (*h*)

between the exospore and endospore shows signs of dissolution; *c*, spore membrane (includes everything between *a* and *g*); *e*, portion of spore membrane becoming transformed into exospore; *g*, endospore. ×600.

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[4] LYON, FLORENCE, A study of the sporangia and gametophytes of *Selaginella apus* and *Selaginella rupestris*. BOT. GAZETTE **31**:124–193. *pls. 5–9.* 1901.

[5] BEER, RUDOLPH, The present position of cell-wall research. *New Phytologist* 3:159–164. 1904.

FIG. 8. Small portion of median section of apical region of megaspore more advanced; the protoplast *b* with the endospore *g* on its outer face has expanded until it has come to lie close to the outside *e;* the undifferentiated part *h* of the spore membrane has almost entirely disappeared; *e*, exospore; *g*, endospore (black line); *h*, layer of granular matter which is all that remains of region between *c* and *g* in *fig. 7*. ×60.

FIG. 9. Apical region of median section of older megaspore; the mother cell membrane has disappeared entirely; *b*, protoplast increasing rapidly; *e*, exospore; *g*, endospore (increasing in thickness by successive laminae formed on its inner surface in the vicinity of the nucleus). ×600.

FIG. 10. Portion of apical region of median section of mature megaspore in the region of the nucleus; the protoplast has increased in thickness at the expense of the central vacuole near which the nucleus lies; the protoplasm contains much proteid matter which stains deeply; *e*, exospore; *g*, endospore. ×600.

FIG. 11. Part of median section of female gametophyte after cell walls have begun to form; the proteid granules have increased in number and in size; *e*, exospore; *g*, endospore; *nn*, nuclei. ×600.

PLATE XI. Selaginella Emmeliana.

FIG. 12. Megaspore mother cell in optical median section, after the formation of the spore membrane. ×1250.

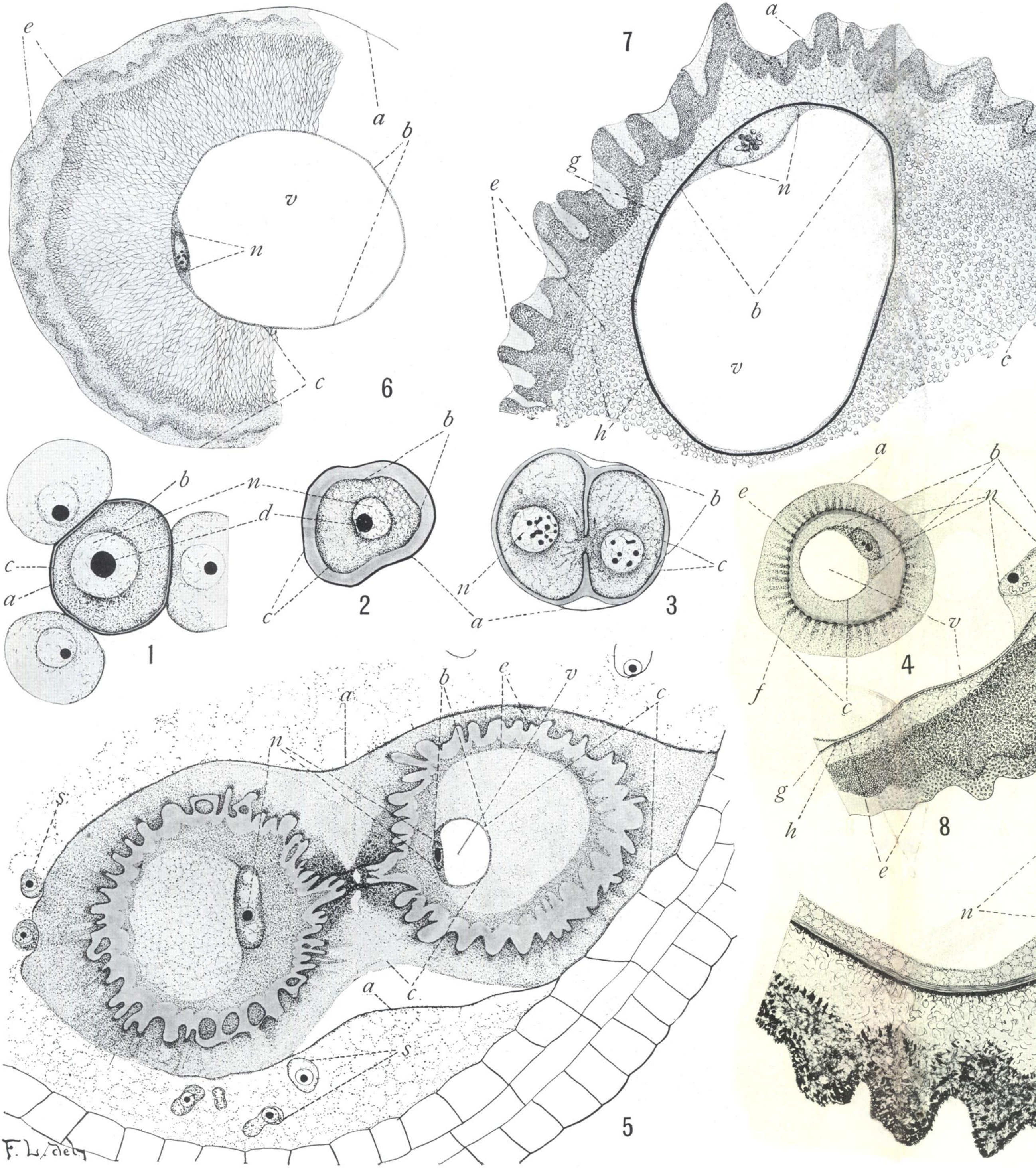
FIG. 13. Median section of megaspore mother cell in process of division into tetrad; nuclear plates forming; *x*, nuclear plate; *y*, spindle fibers. ×1250.

FIG. 14. Median section of megaspore tetrad showing completion of spore membrane along the nuclear plates. ×1250.

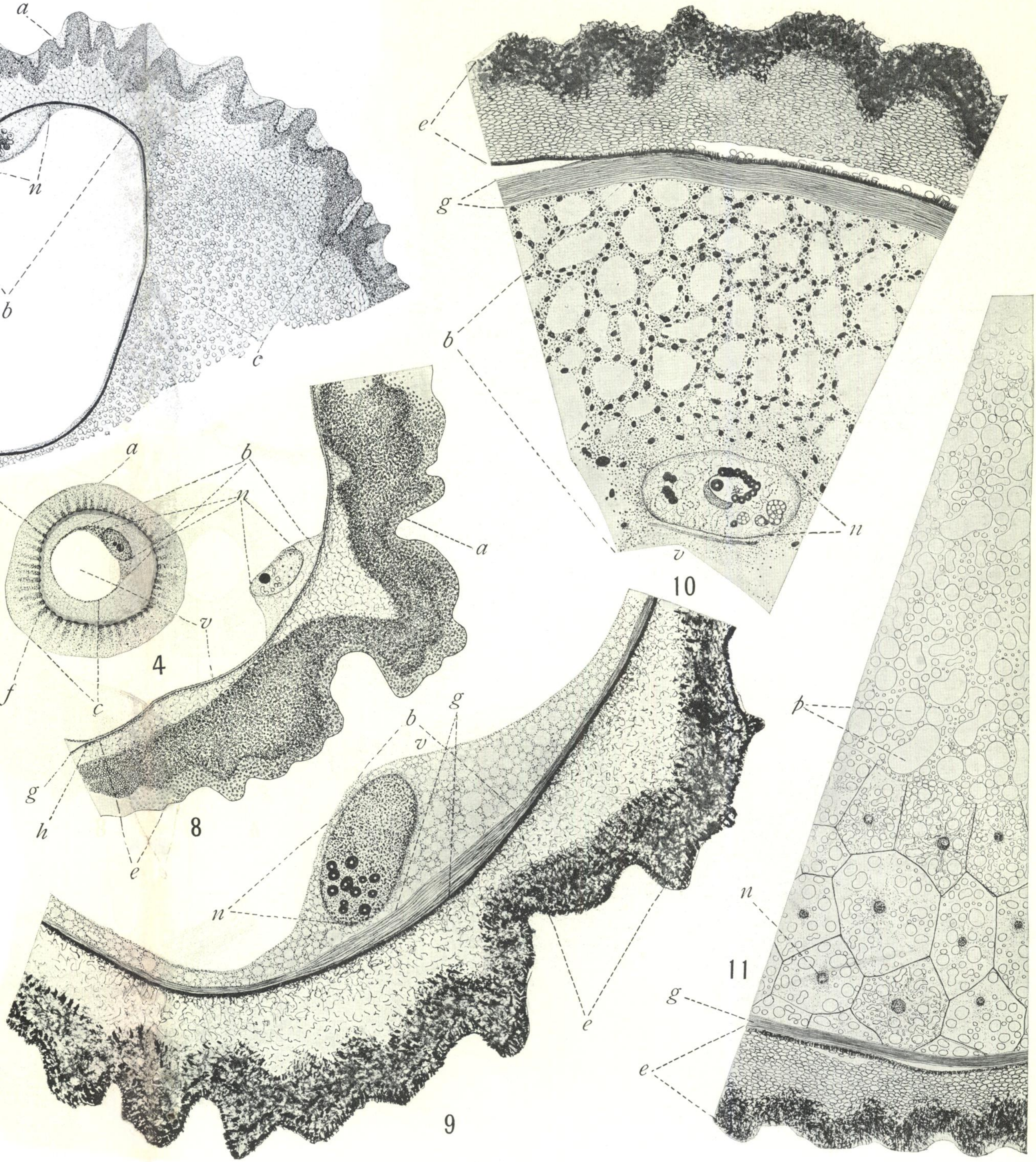
FIG. 15. Median section of somewhat older tetrad, showing increase in thickness of spore membrane between the protoplasts of the spores; protoplasm becoming vacuolate. ×1250.

FIG. 16. Median section of tetrad showing the differentiation of a layer of the spore membrane; the outer region of the shaded band with the convoluted border becomes the exospore, the inner region in contact with the protoplast becomes the endospore; outside of the forming exospore, between it and the mother cell membrane, is a layer of the undifferentiated spore membrane; *d*, portion of spore membrane in process of transformation into coats; *e*, region of exospore; *f*, region of endospore; *m*, undifferentiated portion of spore membrane. ×1250.

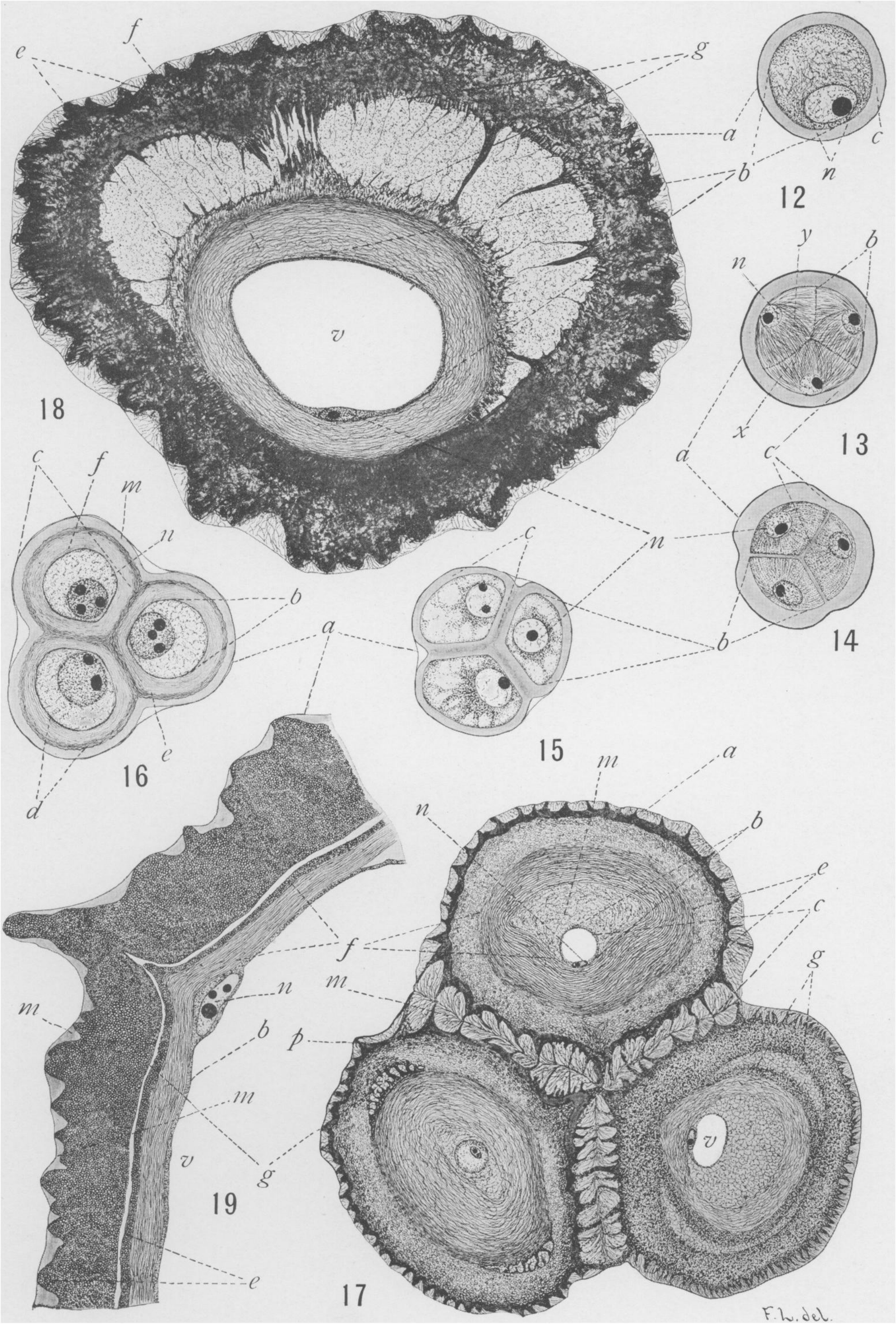
FIG. 17. Nearly median section of tetrad showing farther differentiation of exospore and endospore; two megaspores show the effect of the mechanical strain between exospore and endospore which has a tendency to split the exospore into two layers; stages between *fig. 16* and *fig. 17* were not found among several hundred preparations; the transformation of the spore membrane into coats is



LYON on SPORE COATS OF SELAG



on SPORE COATS OF SELAGINELLA



LYON on SELAGINELLA

very rapid; *e*, exospore; *f*, endospore; *m*, undifferentiated part of spore membrane; *p*, outer sculptured part of exospore (so-called perinium); *g*, strained region in exospore. ×375.

FIG. 18. Nearly median section of a megaspore at a later stage than *fig. 6;* the specimen was slightly shrunken, which gives the appearance of a cleft in the exospore traversed by some unbroken strands; the undifferentiated portion of the spore membrane between the exospore and the protoplast (*fig. 16, m*) has been transformed into endospore; at this stage the protoplast increases in diameter rapidly; *e*, exospore; *f*, endospore; *g*, stretched and partially split region in the substance of the exospore. ×375.

FIG. 19. Small portion of the median section of a nearly mature megaspore in the apical region; coats fully formed; the cleft separating the exospore into two layers is probably due to the action of reagents; *e*, exospore; *f*, endospore; *g*, cleft in substance of exospore; *m*, undifferentiated remnant of spore membrane. ×375.